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# HETEROSYNAPTIC MODULATION OF LONG-TERM POTENTIATION AT MOSSY FIBER SYNAPSES IN HIPPOCAMPUS

AFOSR 88-0142

## Annual Technical Report

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### 1 Summary

The overall goal of this research project is to investigate the cellular and membrane mechanisms associated with the heterosynaptic modulation of long-term synaptic potentiation (LTP) at mossy fiber synapses in the hippocampus. We have previously shown that norepinephrine, through  $\beta$ -adrenoceptors, *enhances* the magnitude, duration, and probability of induction of mossy fiber LTP, while acetylcholine, through muscarinic receptors, *depresses* the magnitude and probability of induction of mossy fiber LTP. The goal for the second year of this research project was to test several specific hypotheses for the induction of mossy fiber LTP. Specifically, the hypotheses relate to the possible requirement of postsynaptic calcium entry through voltage-gated calcium channels during the induction LTP. Moreover, we have been investigating the properties and distribution of voltage-gated calcium channels in hippocampal neurons and the modulation of these calcium channels by noradrenergic and cholinergic agonists. In a collaborative project with Dr. David Terrian, originally at the USAFSAM in San Antonio and now at East Carolina University, the mechanisms of neurotransmitter release from a homogeneous fraction of mossy fiber synaptosomes have been investigated. Taken together, steady and significant progress has been made in a number of directions, all of which are associated with our attempt to understand the mechanisms of excitatory synaptic transmission in the mammalian central nervous system and the neuromodulation of LTP by several neurotransmitters.

### 2 Research Objectives

The research objectives for the funding period 1 April 1989-31 March 1990 were as follows:

- Test the hypothesis that the membrane potential of postsynaptic CA3 neurons is a variable for the induction of LTP.
- Test the hypothesis that multiple types of voltage-gated calcium channels are present in the cell bodies and dendrites of hippocampal neurons.
- Test the hypothesis that norepinephrine, through  $\beta$ -adrenergic agonists, enhances the activity of voltage-gated calcium channels in CA3 neurons.

- d) Test the hypothesis that muscarinic cholinergic agonists depress voltage-gated calcium channels on CA3 neurons.
- e) Test hypotheses for the mechanism of presynaptic autoregulation at mossy fiber synapses.
- f) Investigate the types of calcium channels present in presynaptic mossy fiber terminals.
- g) Develop a single neuron computer model for simulating postsynaptic calcium gradients as a substrate for the induction of LTP.

### 3 Status of Research

#### 3.1 Test the hypothesis that the membrane potential of postsynaptic CA3 neurons is a variable for the induction of LTP.

At most synapses in the hippocampus, activation of NMDA-type glutamate channels is required for the induction of LTP (Collingridge, Kehl and McLennan 1983). It has been hypothesized that calcium influx through these NMDA channels is the requisite first step in the induction of LTP (MacDermott *et al.* 1986; Malenka *et al.* 1988). In contrast, LTP at mossy fiber synapses is *independent* of the activation of NMDA receptors (Harris and Cotman 1986; Williams and Johnston 1988), and there is a very low density of NMDA receptors in the vicinity of these synapses (Monaghan and Cotman 1985). It is obvious, therefore, that some other mechanism must be involved in the induction of mossy fiber LTP. We have proposed, based on previous work, that activation of postsynaptic voltage-gated calcium channels by the high frequency stimulation normally used to induce LTP (and the subsequent calcium entry through these calcium channels) provides an alternate mechanism for LTP at mossy fiber synapses (Hopkins and Johnston 1988). During the first year of this research project we tested one aspect of this hypothesis by demonstrating that the induction of mossy fiber LTP was blocked by the postsynaptic injection of the calcium chelators BAPTA and QUIN-2 (Williams and Johnston 1989).

The second prediction of this calcium channel hypothesis for the induction of LTP is that the membrane potential of the postsynaptic neuron during high frequency stimulation of the mossy fibers should be a variable for the induction of LTP. In other words, hyperpolarization below threshold for activation of voltage-gated calcium channels during high frequency stimulation should block the induction of LTP. Furthermore, depolarization during high frequency stimulation should enhance the magnitude and probability of induction of LTP. During the past year we performed such experiments and the results appear to support the hypothesis. We found that hyperpolarization blocked mossy fiber LTP, and depolarization enhanced LTP. The results suggest that mossy fiber LTP follows a Hebbian rule for induction—concurrent pre- and postsynaptic activity are required for the plasticity to occur. This work has recently been accepted for publication (Jaffe and Johnston 1990a).

#### 3.2 Test the hypothesis that multiple types of voltage-gated calcium channels are present in the cell bodies and dendrites of hippocampal neurons.

It has been reported in a number of preparations that at least three types of voltage-gated calcium channels exist in neurons (Miller 1987). In our studies of voltage-gated calcium channels in granule cells, we obtained preliminary evidence that there were three types (T, N, and L) (Gray and Johnston 1986). We wanted to test the hypothesis that these three types of calcium channels were present in CA1 and CA3 pyramidal neurons, to explore the distinguishing characteristics of the three types of channels, and to determine the relative distribution of these channels among the different hippocampal neurons. Figures 1–3 illustrate the differences in the three types of calcium channels

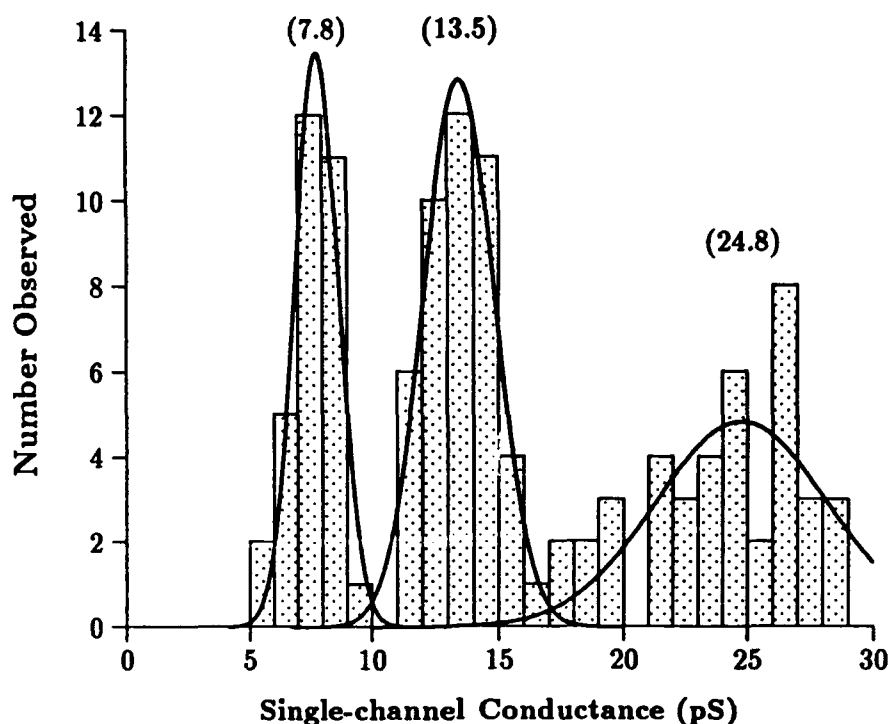


Figure 1: Frequency distribution of 115 single-channel conductances pooled from CA3 and CA1 pyramidal neurons and dentate granule cells. The distribution was fit simultaneously with the sum of three Gaussian curves. The mean value (and standard deviation) for each peak was 7.8 (0.9), 13.5 (1.4), and 24.8 pS (3.4). Binwidth of the distribution was 1 pS.

observed in hippocampal neurons. The T channel has the smallest single channel conductance, it inactivates rapidly with depolarization, and it has the lowest threshold for activation. The L channel, in turn, has the largest single channel conductance, it inactivates relatively little with prolonged depolarization, and it has the highest threshold for activation. The N channel has characteristics that fall in the middle of the T and L. We also found that there was a heterogeneous distribution of the three channel types among hippocampal neurons (see Fig. 4). For example, granule cells contain mostly N-type channels, CA1 neurons contain N- and L-type but fewer T-type than CA3, while all three channel types are abundant on CA3 neurons. The different distribution of channel types among the neurons may help explain their different functional properties. For example, the T-type channel has been suggested to underlie endogenous burst behavior in neurons (Llinás 1988) and the relative burst tendency of hippocampal neurons certainly follows our observed distribution of T-type channels (*i.e.*, granule cells < CA1 < CA3). This work on the properties of calcium channels in the hippocampus has recently been accepted for publication (Fisher, Gray and Johnston 1990).

### 3.3 Test the hypothesis that norepinephrine, through $\beta$ -adrenergic agonists, enhances the activity of voltage-gated calcium channels in CA3 neurons.

With the characterization of at least three types of calcium channels in CA3 pyramidal neurons, it became feasible to test the hypothesis that norepinephrine, through  $\beta$ -adrenoceptors, modulates specific types of calcium channels. Briefly, we found that isoproterenol enhanced the activity of the N- and the L-type channels with essentially no effect on the T-type channel. A summary of the results are illustrated in Figure 5 and a complete manuscript has recently been accepted for

publication (Fisher and Johnston 1990).

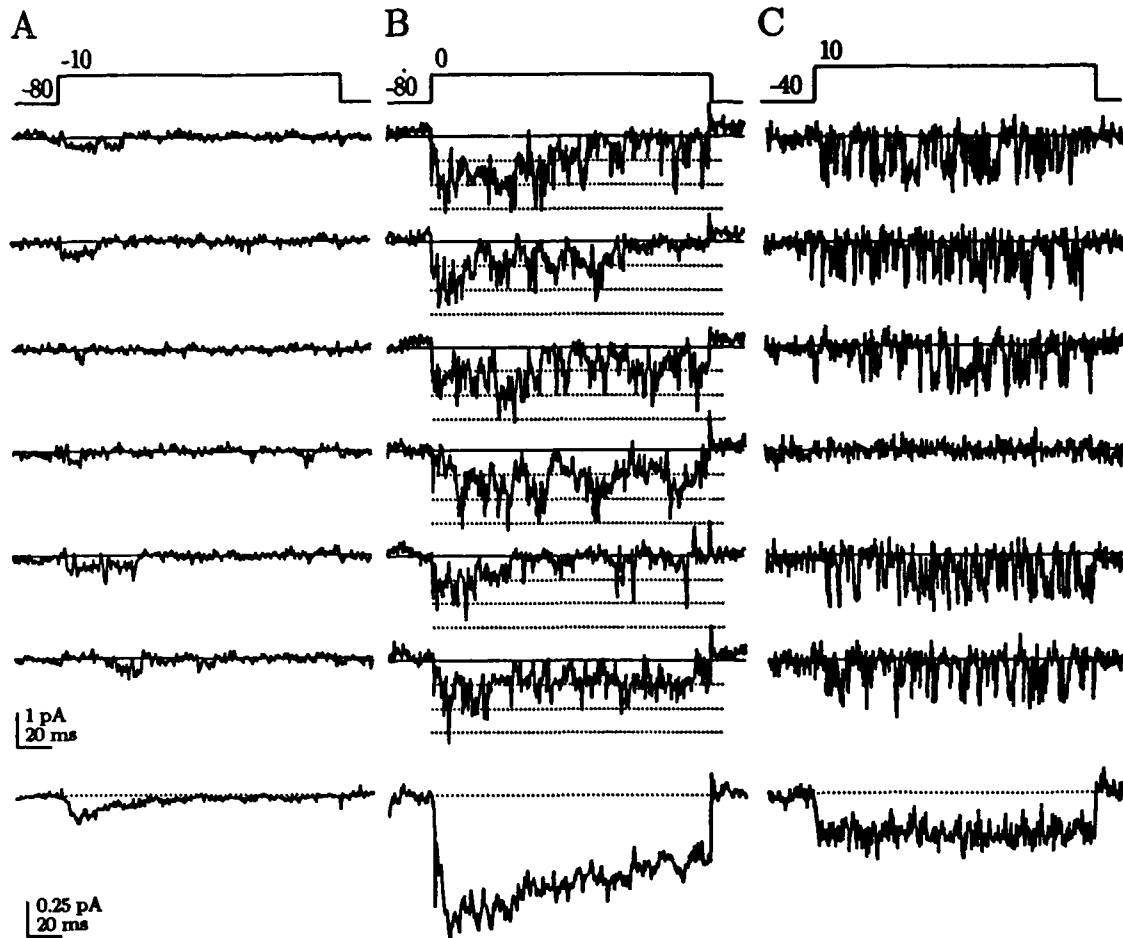


Figure 2: Ensemble averages reveal differences in inactivation of the three types of channels. Single-channel records and ensemble averages from three different patches. **A.** traces from a patch that contained only the 8 pS channel, showing complete channel inactivation during the command step (average of 128 traces). **B.** a patch that contained only 14 pS channels shows considerable inactivation during the command step (average of 41 traces). **C.** a patch that contained only 25 pS channels shows no significant inactivation during the command step (average of 77 traces).

### 3.4 Test the hypothesis that muscarinic cholinergic agonists depress voltage-gated calcium channels on CA3 neurons.

An obvious hypothesis that we derived from our finding that muscarine depresses mossy fiber LTP is that muscarinic agonists might depress voltage-gated calcium channels. We have investigated the effects of carbachol and muscarine on the three types of voltage-gated calcium channels observed in hippocampus. The results are quite interesting. We found that muscarinic agonists depress the L-type, have no effect on the N-type, and increase the T-type calcium channel (see summary in Fig. 6). These results have recently been accepted for publication (Fisher and Johnston 1990). One interesting speculation derived from these results, and those mentioned above, is that the L-type calcium channel may be involved in the induction of mossy fiber LTP. We hope to pursue this idea further during the next year of funding.

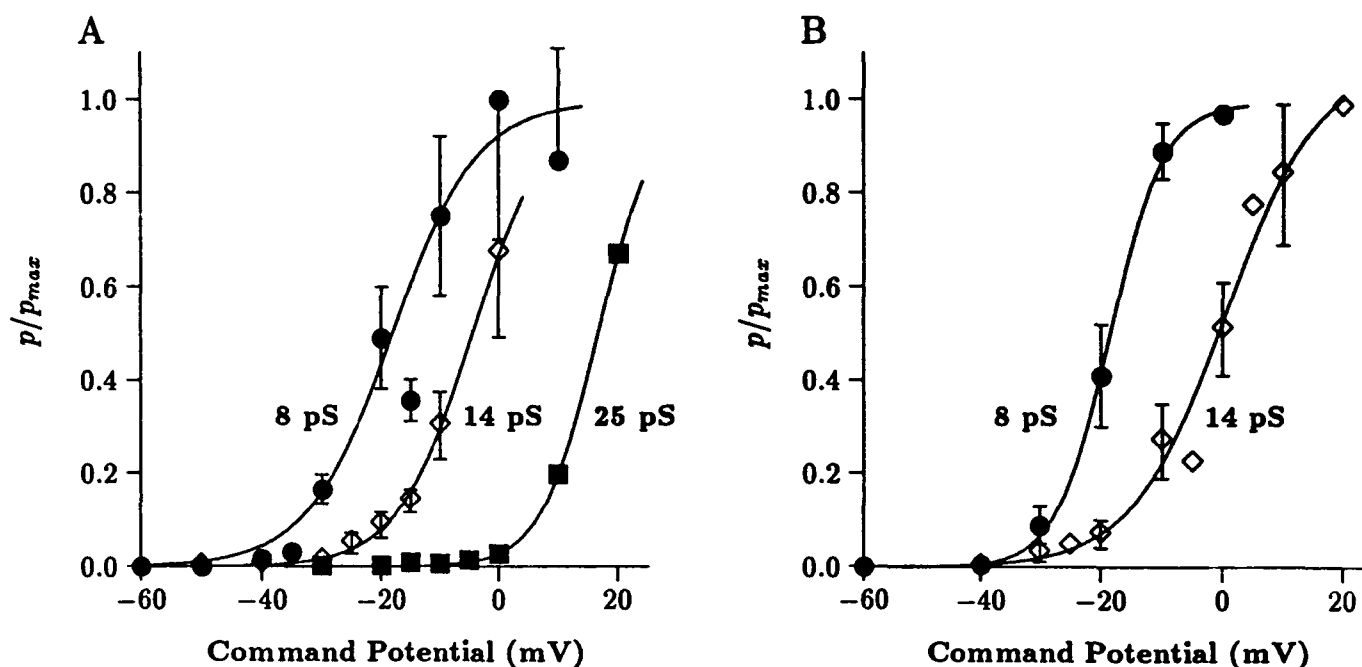


Figure 3: The three channel types show differences in voltage range of activation.  $p$  = probability of channel being in the open state (fractional open time) during the command step;  $p_{max}$  = maximal probability of channel opening; curve is a fitted sigmoid (for details, see METHODS). For the 8 and 14 pS channel data, the patches were held at  $-80$  mV and stepped to the indicated command potentials. For the 25 pS curve, patches were held at  $-40$  mV. A. Activation curves derived from patches from CA3 neurons.  $V_{1/2}$  (half-activation points) and  $k$  (steepness) for each of the curves are:  $-18$  mV, 7.2 (8 pS);  $-4$  mV, 6.3 (14 pS); and 17 mV, 4.7 (25 pS). Each curve is the normalized average of several experiments: 10, 8, and 3 for the 8, 14, and 25 pS channels, respectively. B. Activation curve for the 8 pS channel derived from data from 2 granule and 2 CA1 neurons, and for the 14 pS channel from 3 granule neurons. For the 8 pS curve,  $V_{1/2} = -19$  mV,  $k = 4.3$ ; for the 14 pS curve,  $V_{1/2} = 0$  mV,  $k = 7.7$ .

### 3.5 Test hypotheses for the mechanism of presynaptic autoregulation at mossy fiber synapses (Dr. David Terrian).

During this reporting period, we have further characterized the biochemical properties of isolated hippocampal mossy fiber synaptosomes and continued our investigation of the autoregulation of neurotransmitter release from these large nerve endings. Four specific hypotheses were tested concerning the biochemical basis for mossy fiber synaptic transmission and its presynaptic modulation.

1. The first hypothesis was that the excitatory mossy fiber synaptic input is mediated by both opioids and acidic amino acids. We had previously demonstrated that depolarized mossy fiber synaptosomes release both dynorphin B and endogenous glutamate in a  $Ca^{2+}$ -dependent manner (Terrian et al., 1988). However, the biochemical identity of the mossy fiber neurotransmitter had not been resolved and aspartate, in particular, was considered to be a legitimate candidate. Therefore, experiments were conducted to determine what relative amounts of prodynorphin-derived peptides and endogenous amino acids are concomitantly released from hippocampal mossy fiber synaptosomes.

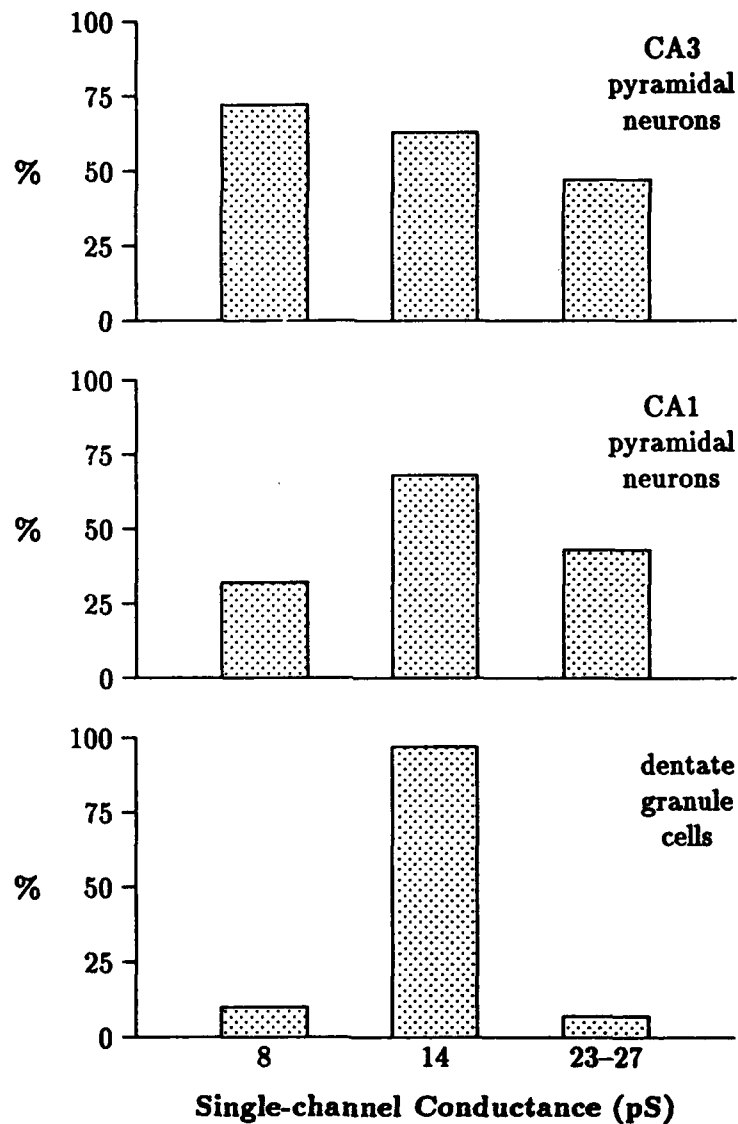


Figure 4: Relative distribution of the three channel types in each of the three principal cell types of the hippocampal formation. Each channel measured was classified as small (8 pS), medium (14 pS), or large (25 pS) conductance and binned accordingly. A. CA3 neurons. Distribution of small and medium-conductance channels taken from 32 patches; distribution of large-conductance channel taken from 64 patches. B. CA1 neurons. Distribution taken from 28 patches. C. granule cells. Distribution from 29 patches.

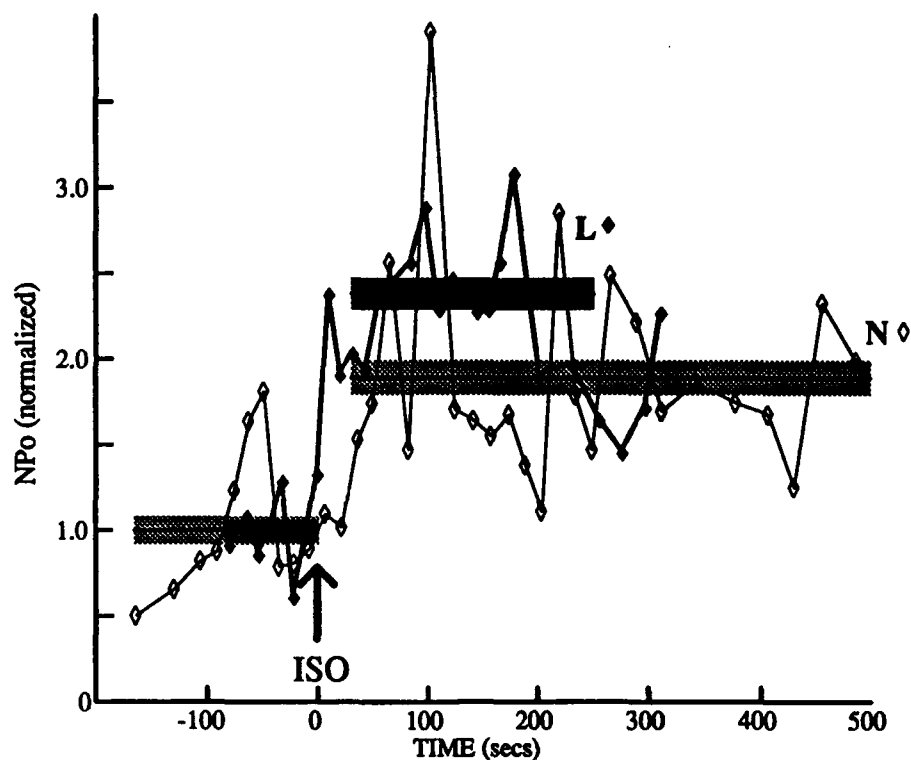


Figure 5: Summary of isoproterenol effects on all patches containing N and L channels. Filled diamonds represent averaged data points for all experiments on L channels; darkly shaded boxes are mean  $\pm$  sem before and after isoproterenol application to L channels. Unfilled diamonds represent averaged points for all experiments on N channels; lightly shaded boxes are mean  $\pm$  sem before and after isoproterenol application to N channels. Average changes in  $NP_o$  were as follows: 89% increase (Ns, 7 experiments) and 138% increase (Ls, 6 experiments). Plots of probability of channel opening *vs* time for each individual experiment were normalized for both x and y axis values (so that, for each experiment, drug application occurred at time = 0 seconds and the average pre-drug value of  $NP_o$  was 1.0). All normalized data points for all experiments on N (or L) channels were then pooled. Every 30 data points were averaged and plotted as one point in this figure.



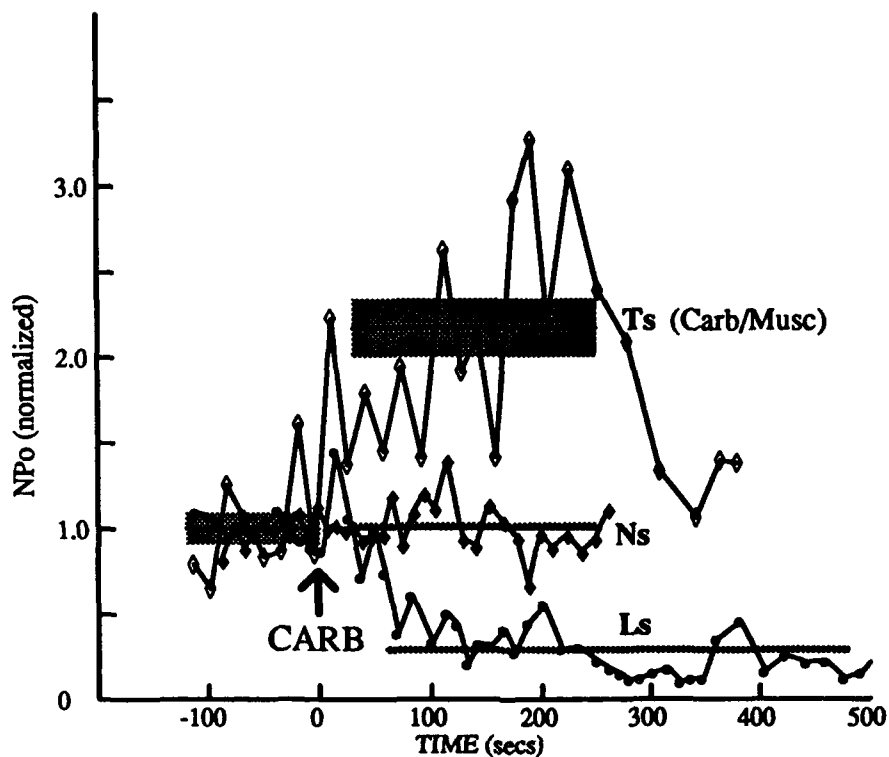


Figure 6: Summary of carbachol effects on all patches containing T and L channels. Unfilled diamonds represent averaged data points for all experiments on T channels, filled diamonds for N channels, and filled circles for L channels. Shaded boxes are mean  $\pm$  sem before and after carbachol application. The post-drug variation for N and L channels was too small for the shaded boxes to be clearly visible. The pre-drug shaded box is for carbachol on T channels; the corresponding values for N and L channels showed less variability. Average changes in  $NP_o$  were as follows: 117 % increase (Ts, 6 experiments), 1 % increase (Ns, 9 experiments), and 71 % decrease (Ls, 7 experiments). The plot for T channels includes both carbachol and muscarine data. Normalization and plotting of data are the same as described in Fig. 5 legend.

2. The second hypothesis was that a presynaptic excitatory amino acid receptor autoregulates the release of neurotransmitters from mossy fiber terminals. Preliminary evidence to support this hypothesis was reported in our previous Annual Technical Report, where it was demonstrated that the glutamate analogue L(+)-2-amino-4-phosphonobutyric acid (APB) suppressed the evoked release of both glutamate and dynorphin from mossy fiber synaptosomes. During the present reporting period additional experiments were conducted to examine the mechanism of this presynaptic autoregulation and to identify the type of receptor that is involved.
3. The third hypothesis was that a presynaptic opioid autoreceptor also modulates the release of mossy fiber neurotransmitters. Immunocytochemical and autoradiographic studies had previously been able to provide indirect support for such a presynaptic receptor, but no direct biochemical evidence was available.
4. The fourth hypothesis was that the presynaptic mechanism(s) underlying the maintenance of LTP in the mossy fiber synapse does *not* involve the activation of protein kinase C. It has been proposed that the maintenance of LTP in the mossy fiber synapse involves fundamentally different presynaptic mechanisms from those employed by other hippocampal synapses, since both protein kinase C and phosphoprotein F1 (GAP-43) appear to be absent in mossy fiber terminals. Using the mossy fiber synaptosomal preparation, it was possible to directly test this hypothesis.

The results of the experiments that were conducted during this past year to test the first two hypotheses have either been published (Gannon, Baty and Terrian 1989; Gannon and Terrian 1989; Terrian, Gannon and Rea 1990) or have been submitted for publication (Gannon and Terrian 1990; Terrian, Dorman and Gannon 1990). The later two hypotheses were tested by Dr. Terrian more recently, following his relocation to the East Carolina University School of Medicine, and have not yet been submitted for publication. The results of these experiments can be summarized as follows:

1. Of the 18 amino acids shown to be present in superfusate fractions by liquid chromatographic analysis, only glutamate was released at a significantly enhanced rate from depolarized mossy fiber nerve endings. The release of glutamate and aspartate was increased by  $360 \pm 27\%$  and  $54 \pm 12\%$  over baseline, respectively. However, the evoked release of glutamate was substantially more  $\text{Ca}^{2+}$ -dependent (80%) than was the release of aspartate (49%). Depolarization also stimulated the release of the four prodynorphin (Dyn) products examined, in a rank order of Dyn B >> Dyn A(1-17) > Dyn A(1-8) >> Dyn A(1-13), with Dyn B efflux increasing by more than 5-fold over baseline values. These results suggest that the predominant excitatory amino acid in hippocampal mossy fiber synaptic transmission may be glutamate and that this synaptic input may be modulated by at least four different products of prodynorphin processing.
2. Excitatory amino acid agonists and antagonists were evaluated for their ability to affect the concomitant release of endogenous glutamate and Dyn A(1-8) from guinea pig mossy fiber synaptosomes. Low micromolar concentrations of quisqualate, but not kainate, *N*-methyl-D-aspartate (NMDA), nor RS- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, significantly inhibited the depolarization-evoked release of both glutamate and Dyn A(1-8). Quisqualate-induced inhibition of glutamate release from mossy fiber terminals was antagonized by the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In contrast, high concentrations of kainate enhanced the evoked release of glutamate and Dyn A(1-8), and this potentiation was blocked by CNQX. These results suggest a bimodal mechanism for the autoregulation of neurotransmitter release from mossy fiber terminals. Previous

reports have indicated that the presynaptic kainate receptors may contribute to the unusual sensitivity of the mossy fiber-CA3 pathway to epileptic damage and that these receptors may only become fully expressed or active during the reactive synaptogenesis that occurs following hippocampal neuronal damage.

3. We have recently examined the effects of the  $\kappa$  agonist U50488H on calcium availability and the release of glutamate and Dyn B from mossy fiber synaptosomes. The results of these studies demonstrated that U50488H produces a dose-dependent inhibition of the depolarization-induced rise in cytosolic free calcium and the release of both neurotransmitters. The estimated  $IC_{50}$  value for this effect is  $30 \mu M$ . The inhibitory effect of U50488H was also reversed by the selective  $\kappa$  antagonist nor-binaltorphimine. These results suggest the existence of a  $\kappa$  opioid autoreceptor capable of modulating mossy fiber synaptic transmission.
4. The metabolic properties of hippocampal mossy fiber synaptosomes were compared to those of a conventional  $P_2$  synaptosomal fraction prepared from the same hippocampal tissue. Protein kinase C-dependent histone phosphotransferase activity was found to be comparable in mossy fiber and  $P_2$  synaptosomes. Western blot analyses were performed to confirm this unexpected finding, and the results demonstrate that the  $\alpha$ ,  $\beta$ , and  $\gamma$  subspecies of protein kinase C are all present in relatively equivalent amounts in these two different subcellular fractions. However, an SDS-PAGE analysis of the endogenous substrates phosphorylated by protein kinase C indicated that protein F1 is not present in mossy fiber nerve endings. A functional role for protein kinase C in the mossy fiber terminal seems to be indicated by the finding that phorbol-12,13-dibutyrate and phorbol-12,13-diacetate produce a dose-dependent potentiation of the  $K^+$ -evoked increase in the availability of cytosolic free calcium and the concomitant release of endogenous glutamate and Dyn B. The biologically inactive 4- $\alpha$ -phorbol was without effect on any of these parameters, and the phorbol-12,13-dibutyrate ( $1 \mu M$ ) enhancement of  $Ca^{2+}$ -dependent release was blocked by the protein kinase C antagonist staurosporine ( $1 \mu M$ ). Based on these results, we conclude that hippocampal mossy fiber nerve endings possess a variety of protein kinase C isoforms and that their activation is sufficient to have an important influence on mossy fiber synaptic transmission and plasticity.

### 3.6 Investigate the types of calcium channels present in presynaptic mossy fiber terminals.

During the past year, we have continued our investigation of presynaptic calcium channels at mossy fiber synapses. Once again the results are tantalizing yet incomplete. Richard Gray, a co-investigator on this project, spent a month in the laboratory of John Connor to use calcium imaging techniques for the visualization of intracellular calcium from isolated mossy fiber terminals. During this collaboration, they were able to demonstrate a potassium stimulated increase in intraterminal calcium, but unfortunately the increased calcium persisted even after the return to normal extracellular potassium, suggesting an abnormal buffering of intraterminal calcium in the isolated terminals. Rick has also successfully measured whole-cell calcium currents from presynaptic terminals using nystatin and the perforated patch technique (Korn and Horn 1989), but the experiments have not succeeded often enough to yield useful data. We have therefore decided to publish the results that we have accumulated over the past few years (outlined in last year's Annual Report) rather than waiting (and hoping) for a technical breakthrough. The results certainly don't represent a complete story, but nevertheless are interesting for those trying to understand presynaptic calcium channels and their role in transmitter release. It is a difficult problem, and we have probably made more progress than most others in the field, but we are still somewhat disappointed that we have been unable to go further. We do, however, have a number of new ideas

that we hope to try during the upcoming funding period.

### 3.7 Develop a single neuron computer model for simulating postsynaptic calcium gradients as a substrate for the induction of LTP.

Although not part of the original grant application, we have continued to develop and use single cell computer models to simulate various hypotheses related to mossy fiber synaptic transmission and LTP. Using our recent data for different types of calcium channels, we have constructed a fairly realistic model of CA3 pyramidal neurons. We are interested in the calcium influx through calcium channels during synaptic stimulation and the buffering of this calcium in different regions of the neuron (e.g., spine head, spine shaft, and apical dendrite). The results of the simulations have suggested a possible explanation for why, in a preliminary study by German Barrioneuvo, intracellular injection of EGTA did not block mossy fiber LTP. It turns out that the amount of calcium influx that occurs through voltage-gated calcium channels during high frequency synaptic stimulation is so high that the buffering capacity of EGTA (but not BAPTA) is exceeded. We will be presenting this work at this year's Society for Neuroscience annual meeting (Jaffe and Johnston 1990b).

## 4 Publications

### 4.1 Full papers and review articles

1. Hopkins, W.F. and Johnston, D. Noradrenergic modulation of synaptic plasticity in the hippocampus. In: *Problems and Concepts in Developmental Neurophysiology*. Kellaway, P. and Noebels, J.L., (eds.), Johns Hopkins Univ. Press: Baltimore, 1989, pp. 92-109.
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5. Claiborne, B.J., Rea, M.A., and Terrian, D.M. Detection of zinc in isolated nerve terminals using a modified Timm's sulfide-silver method. *J. Neurosci. Methods* 30:17-22, 1989.
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11. Gray, R., Fisher, R., Spruston, N., and Johnston, D. Acutely exposed hippocampal neurons: A preparation for patch clamping neurons from adult hippocampal slices. In: *In Vitro Preparations From Vertebrate Nervous Systems*. Jahnsen, H. (ed.), John Wiley: England, (in press).
12. Jaffe, D.B. and Johnston, D. The induction of long-term potentiation at hippocampal mossy fiber synapses follows a Hebbian rule. *J. Neurophysiol.* (in press).
13. Williams, S. and Johnston, D. Muscarinic depression of synaptic transmission at the hippocampal mossy fiber synapse. *J. Neurophysiol.*, (in press).
14. Fisher, R., Gray, R., and Johnston, D. Properties and distribution of single voltage-gated calcium channels in adult hippocampal neurons. *J. Neurophysiol.*, (in press).
15. Fisher, R. and Johnston, D. Differential modulation of single voltage-gated calcium channels by cholinergic and adrenergic agonists in adult hippocampal neurons. *J. Neurophysiol.*, (in press).
16. Johnston, D., Fisher, R.E., and Gray, R. Voltage-gated calcium channels in adult hippocampal neurons. In: *Ion Channels, Volume III*. Narahashi, T., (ed.), Plenum Publishing Corp: New York, (in press).
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#### 4.2 Abstracts

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## 5 Professional Personnel Associated With the Research Project

Daniel Johnston, Ph.D.—Principal Investigator  
 Richard A. Gray, Ph.D.—Co-investigator  
 Ron Fisher—Graduate Student (graduated 12/89)  
 David Jaffe—Graduate Student  
 Nelson Spruston—Graduate Student  
 Mahmud Haque—Computer Systems Manager  
 Judy Walker, M.S.—Research Technician  
 David Terrian—Co-investigator  
 Anna Marie Michel—Research Associate

## 6 Interactions

- 4/8/89 Premedical Advisor Workshop
- 5/08/89- Mahmud Haque to Masscomp User's Society, Boston, MA  
5/14/89
- 6/23/89 Seminar for the Division of Neuroscience, Baylor. "Mechanisms of LTP in hippocampus."
- 6/29/89- NIMH Study Section, Washington, DC  
6/30/89
- 7/18/89- Study Section "MHK Research Scientist Development," Washington, DC  
7/19/89
- 8/04/89 Lecture for SMART students at Baylor
- 10/05/89 NIH-MBRS Symposium
- 10/29/89- Society for Neuroscience, Phoenix, Arizona  
11/02/89
- 12/10/89- Study Section, Special Review Committee  
12/11/89
- 2/06/90 Lecture to MSTP students at Baylor
- 3/02/90 Seminar at the University of Texas Medical Branch in Galveston for the Department of Pharmacology. "NMDA independent LTP in hippocampus."
- 3/29/90- Study Section, Special Review Committee  
3/30/90

## 7 New Discoveries, Inventions, or Patent Applications

None.

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